VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

The following paragraph and heading has been added, immediately following the title:

Cross-Reference to Related Applications

This is the U.S. national phase under 35 U.S.C. § 371 of International Application Number PCT/EP00/08071, filed August 18, 2000 which claims priority to European Application Number 99116766.9, filed August 30, 1999 under 35 U.S.C. § 119, the disclosures of which are incorporated herein by reference.

The following heading has been added following the section entitled "Cross-Reference to Related Applications":

Field of the Invention

The paragraph beginning on page 1, line 3, has been deleted and rewritten as follows:

[The present invention relates to a mouse parkin2 DNA- and protein sequence containing naturally occurring or artificially introduced mutations or deletions, which cause Parkinson's disease in a human if they occur in the according human sequence, the construction of a truncated parkin gene, which expresses no, a non-active or a truncated parkin protein and a model of a transgenic animal, expressing such a less or non-active parkin protein instead of the native parkin protein or no parkin protein, as well as to the use of such a transgenic animal as a model for neurodegenerative diseases, preferred Parkinson's disease.] This invention relates to a transgenic animal model containing mutated mouse parkin2 DNA and translated protein sequence. The use of a transgenic animal can be used as a model for neurodegenerative diseases, preferably Parkinson's disease.

The following heading has been added prior to the paragraph beginning at page 1, line 13:

Description of the Related Art

The paragraph beginning at page 1, line 13 has been amended as follows:

Neurodegenerative disorders are some of the most feared illnesses in society. During the last 10 years some of the genetic causes of many of the primary neurodegenerative disorders like Alzheimer's disease, Parkinson's disease, Huntington´s disease, amyotrophic lateral sclerosis, prion disease and several ataxic syndromes, have been identified. These findings gave new [insults] insights in the knowledge about the initiating trigger as well as the resulting

consequences of those diseases. Due to the fact that these diseases have many pathological mechanisms in common it seems possible that only relatively few pathways to neuronal death are involved in these disorders. Thus, treatment strategies for a particular neurodegenerative disease may be found to have value in other related disorders.

The paragraph beginning on page 2, line 8, has been amended as follows:

In both the early and late onset types of Parkinson's disease (PD), the pathology is the same but the abnormalities tend to be more severe and more widespread in cases beginning at an earlier age. The disease is characterised by lesions in brain areas where the cell bodies of the dopaminergic neurons are located mainly in the substantia nigra compacta. In addition intracytoplasmic inclusions known as Lewy bodies can be observed in different brain regions, in particular in substantia nigra and the locus ceruleus.

The following heading and paragraph has been added prior to the paragraph beginning at page 4, line 9 as follows:

Summary of the Invention

The present invention relates to a mouse parkin2 DNA- and protein sequence containing naturally occurring or artificially introduced mutations or deletions, which cause Parkinson's disease in a human if they occur in the according human sequence, the construction of a truncated parkin gene, which expresses no, a non-active or a truncated parkin protein and a model of a transgenic animal, expressing such a less or non-active parkin protein instead of the native parkin protein or no parkin protein, as well as to the use of such a transgenic animal as a model for neurodegenerative diseases, preferred Parkinson's disease.

The following heading and paragraph have been added following the section entitled "Summary of the Invention":

Brief Description of the Invention

Figure 1 shows the alignment of the deduced amino acid sequences of the human and mouse Parkin2 protein (SEQ ID NO: 4).

Underlinded are the conserved ubiquitin like (at the N-terminus) and Ring finger like (at the C-terminus) regions of both proteins.

Figure 2 shows the alignment of the nucleotide sequences of the human and mouse parkin 2 gene. Bold lines represent the exon boundaries identified for the human and mouse sequence.

Figure 3 represents a flow chart of the cloning procedure of the mouse parkin2 gene - exon3 knock-out construct.

The following heading has been added following the section entitled "Brief Description of the Invention."

Description of the Drawings

The paragraph beginning at page 5, line 17, has been amended as follows:

The transgenic non-human animals according to the present invention can be used as models for [analysing] analyzing the symptoms of neurodegenerative diseases or as a model system for testing the efficacy of a treatment for a neurodegenerative disease[, whereby it is not an object of the present application to provide any method for treating one of the described diseases in a human or animal].

The paragraph beginning at page 7, line19, has been amended as follows:

"Homologous amino acid sequence" in [content] context of with the mouse parkin2 protein means in the present application an amino acid sequence, wherein at least 70 %, preferably 80 %, more preferably 90 % of the amino acids are identical to one of the proteins of the present invention and wherein the replaced amino acids preferably are replaced by homologous amino acids. As "homologous" amino acids are designated which have similar features concerning hydrophobicity, charge, steric features etc. Most preferred are amino acid sequences, containing the species-dependent differences of the mouse amino acid sequence compared to human parkin protein shown in the alignment Figure No. 1. The alignment of the corresponding polynucleotide sequences with the exon boundaries is shown in Figure No. 2.

The paragraph beginning at page 8, line 2, has been amended as follows:

In the whole application for nucleotides and amino acids the usual designations (one-letter [ore] or three-letter code) are used, known by any person skilled in the art.

The paragraph beginning at page 9, line 17 has been amended as follows:

To obtain at least a transgenic non-human animal as a model for neurodegenerative diseases, the natural occurring sequence of the parkin gene in this animal may be replaced on one or both alleles of the chromosomes by a sequence of mPark2, containing mutations or deletions according to the present invention. These animals produce either less or [less] more active or no parkin protein.

The paragraph beginning at page 9, line 28, has been amended as follows:

[Briefly, a] A vector is constructed that carries the replacement DNA. Both ends of the replacement DNA are flanked by long DNA sequences homologous to the sequences flanking the target DNA. When the vector is introduced into ES cells, the homologous sequences align and recombination may take place. This results in the target DNA being exchanged for the replacement DNA. If the [The] vector is not replicated in the cells, it [and] will be lost. The frequency of homologous recombination is low; thus, a screening system is used. The replacement DNA will contain a positive marker sequence, usually a neomycin resistance gene. Thus, any cells that incorporate the replacement DNA by homologous recombination will resist neomycin. By growing cells in medium containing the drug neomycin one can select only those cells containing the replacement DNA. The ES cells containing the replacement DNA are then inserted into recipient mouse blastocysts to create chimeric mice. Chimeras with germ cells derived for the altered ES cells transmit the modified genome to their offspring, yielding mice heterozygous for the target DNA (contain one target DNA and one replacement DNA). The heterozygotes are then bred with each other either to create mice homozygous for the replacement DNA and deficient in the target DNA or to maintain transgenic heterozygotes if the homozygotic mice are not viable.

The paragraph beginning on page 16, line 1, has been amended as follows:

Further to the above described techniques a step of expressing the treated sequence may be inserted in the [expiration] expression vector. Therefore the construct is (sub)cloned into any expression vector, which may be brought into a suitable eukaryotic cell. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline resistance or hygromycin resistance, to permit detection and/or selection of those cells transformed with the desired DNA sequences. Polynucleotides encoding a variant parkin2 polypeptide may include sequences that facilitate transcription (expression sequences) and translation of the coding sequences, such that the encoded polypeptide product is produced. Construction of such polynucleotides is well known in the art and is described further in Maniatis et al. Molecular Cloning: A Laboratory Manual, 2nd Ed. (1989), Cold Spring Harbor, N.Y. For example, but not for limitation, such polynucleotides can include a promoter, a transcription termination site (polyadenylation site in eukaryotic expression hosts), a ribosome binding site,

and, optionally, an enhancer for use in eukaryotic expression hosts, and, optionally, sequences necessary for replication of a vector.

The paragraph beginning on page 20, line 17, has been amended as follows:

[The pups will usually be] Offspring are generally born 16-18 days after introduction of the blastocysts into foster mothers. Chimeric animals will be mated with wild type (wt) mice to create heterozygote transgenics.

The paragraph beginning on page 22, line 12, has been amended as follows:

Preferred are the above described polynucleotide sequences, the proteins and amino acid sequences as well as the transgenic animal models and cell lines that may be used for any method for analysing the symptoms of neurodegenerative diseases.

The paragraph beginning on page 26, line 8, has been deleted:

[a) Restriction endonucleases:

N = NotI, E= Eco RI, B= BamHI, H= HindIII, X= XbaI.

b) Modifications: ()= T4 DNA polymerase treatment in order to remove a restiction site in the resulting plasmid.

c) =pBluescript KSII (Stratagene) vector sequence

= λ-Fix vector sequence

d) HSV-tk = herpes simples promotor and thymidine kinase gene

e) kb = kilobases]

IN THE CLAIMS:

Claims 2, 9, 10, 11, 12, 16, 19, 21 have been deleted.

The remaining claims have been amended as follows.

1. (Amended) **[A]** An isolated or purified polynucleotide **[sequence]** encoding a mutant mouse parkin2 protein, or a homolog thereof, wherein said mutant causes **[containing naturally occurring or artificially introduced mutations or deletions, which cause]** Parkinson's disease **[in a human if they occur in the according human sequence]**.

3. (Amended) The **[sequence]** polynucleotide of claim 1 **[or 2]**, wherein **[the sequence]** said polynucleotide is selected from the group**[,]** consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:7 SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ

ID NO:18, and SEQ ID NO:19, SEQ ID NO:20 **[or naturally occurring or artificially introduced mutants or fragments thereof]**.

4. (Amended) A vector, **[containing any sequence according to any]** comprising the polynucleotide of **[claims]** claim 1 **[to 3]**.

5. (Amended) A **[prokaryotic or eukaryotic]** cell, **[containing a vector according to]** comprising the polynucleotide of claim **[4]** 1.

6. (Amended) The cell of claim 5, **[characterised in that]** wherein the cell is **[selected from bacterial or yeast cells, insect cells or mammalian cells as primary cells or immortalised cell lines]**a prokaryotic or eukaryotic cell.

7. (Amended) A parkin mouse protein, comprising any **[with an]** amino acid sequence selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:6 SEQ, ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34 [or naturally occurring or artificially introduced mutants with a homologous protein sequence or fragments thereof].

8. (Amended) A transgenic non-human **[animal]** mammal**[,]** comprising the isolated or purified polynucleotide of claim 1 **[whose one or both alleles of a gene encoding a parkin gene are mutated or truncated in a way, that a protein with modified, preferred less activity or no active protein is expressed]**.

13. (Amended) A mammalian cell-line transformed or transfected with the polynucleotide of claim 1**[any sequence according to any of claims 1 to 3 or a vector according to claim 4 or cell lines or primary cultures derived from the transgenic animal of any of claims 8 to 12]**.

14. (Amended) A method of producing a transgenic animal, comprising: **[according to any of claims 8 to 12 or a cell line according to claim 13.]**

constructing a vector that carries the polynucleotide of claim 1;

introducing said vector into embryonic stem cells;

injecting said embryonic stem cells into blastocysts; and

placing said blastocycsts into pseudopregnant femal animal.

15. (Amended) **[Use of the transgenic non-human animal according to any of claims 8 to 12 or a cell line according to claim 13 as a]** A mammalian model for a neurodegenerative **[diseases]** disease comprising the transgenic mammal of claim 8.

17. (Amended) A method for testing the efficacy of a treatment for a neurodegenerative disease, comprising: **[associated with a less active or non-active parkin protein, comprising subjecting any model of claim 15 to a putative treatment and determining the efficacy of said treatment.]**

subjecting the mammalian model of claim 15 to a putative treatment or agent; and

determining the efficacy of said treatment by identifying a reduction in the symptoms of said neurodegenerative disease.

18. (Amended) The method **[according to]** of claim **[16 or]** 17, wherein said neurodegenerative disease is selected from the group consisting of: Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, Multisystem atrophy, Wilson's disease, Pick's disease, and Prion disease**[, or second causes inducing Parkinson's syndromes like toxins, drugs, brain tumors, head trauma, stroke, vascular irregularities, or metabolic irregularities]**.

20. (Amended) **[Use of any model according to claim 15]** A method for testing whether an active substance is useful for treating the symptoms of Parkinson's disease comprising: **[a condition associated with non-active parkin protein comprising administering said active substance to the transgenic animal of any of claims 8 to 12 or a cell-line of claim 13, and determining a level of the active substance, which causes an effect in treating the disease.]**

administering said active substance to the transgenic animal of claim 8, and

determining whether said active substance reduces the symptoms of Parkinson's disease.

22. (Amended) A descendant **[Descendant]** of the transgenic animal according to **[any of claims]** claim 8 **[to 12]** wherein, said animal is obtained by breeding with the same or any other genotype.

The following claims have been added.

23. (New) The polynucleotide of claim 1, wherein said mutant comprises a point mutation, deletion or fragment.

24. (New) The polynucleotide of claim 1, wherein said homolog is human.

25. (New) The cell of claim 5, wherein said eukaryotic cell is a fungal, insect or mammalian cell.

26. (New) The cell of claim 25, wherein said fungal cell is a yeast cell.

27. (New) The cell of claim 25, wherein said prokaryotic cell is a bacterial cell.

28. (New) The polynucleotide of claim 1, wherein said mutants comprise mutations in exon 1 or exon 3.

29. (New) The mammalian model of claim 15, wherein said animal is a mouse or rat.

30. (New) A method of testing agents for efficacy and toxicity in treating a neurodegenerative disease, comprising:

administering said agent to the mammalian model of claim 15; and

identifying whether said agent reduces the symptoms of said neurodegenerative disease or is toxic to said mammal.

31. (New) A method for testing whether an active substance is useful for treating the symptoms of Parkinson's disease, comprising:

administering said active substance to the cell-line of claim 13; and

determining whether said active substance reduces the symptoms of Parkinson's disease.

32. (New) The method of claim 20, futher comprising testing various dosages of said active substance.